

Estimation of serum malondialdehyde in potentially malignant disorders and post-antioxidant treated patients: A biochemical study

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Abstract

Background: Tobacco causes the generation of free radicals and reactive oxygen species (ROS) which are responsible for the high rate of lipid peroxidation. Malondialdehyde (MDA) is the most widely used agent to estimate the extent of lipid peroxidation. Timely diagnosis of the condition followed by supplementation with antioxidants like beta-carotene, pro-vitamin A, vitamin A, vitamin C, vitamin E, lipoic acid, zinc, selenium, and spirulina can prevent potentially malignant disorders. **Materials and Methods:** In this study, serum MDA was measured according to the method of Buege, in 15 normal samples and 15 patients who were histopathologically diagnosed with potentially malignant disorders and they were prescribed with antioxidants for a period of 4 week-time following which potentially malignant patients serum MDA was analyzed again to determine the extent of peroxidation reactions. **Results:** The mean serum MDA level in Group C1 was $0.7900 \pm 0.2336 \mu\text{M/L}$ were as the mean serum MDA level of Group S1 was $2.478 \pm 0.50756 \mu\text{M/L}$ and the values between them were highly significant. The values between C1 and S2 were found to be statistically significant. The mean serum MDA of S2 was $2.160 \pm 0.41252 \mu\text{M/L}$ and the values were significant when compared to S1. **Conclusion:** Serum MDA estimation in oral pre-cancer would serve in determining the extent of lipid peroxidation. Diagnosis of patients and administration of antioxidants has proven to be effective in declining the ROS and thus reducing the extent of damage on the cells. MDA may serve as a diagnostic tool in the estimation of oral pre-cancer and in evaluation of post-treated cases.

Keywords: Antioxidants, free radicals, lipid peroxidation, malondialdehyde

Introduction

Free radicals and reactive oxygen species (ROS) like hydrogen peroxide and hydroxyl radicals are highly reactive due to the presence of unpaired valence electrons.^[1] Reactive free radicals are able to produce chemical modifications and damage lipids, proteins, carbohydrates, and nucleotides in the tissues.^[2] As the cell membrane is high in polyunsaturated fatty acids this makes them susceptible to the free radical attack and thus affects the hemostatic environment. Lipid peroxidation is a chain reaction that produces free radicals that cause lipid peroxidation.^[3] When pro-oxidants increase,

antioxidants fail, a situation of oxidative stress caused by the imbalance between the ROS and the body biological ability to detoxify the reactive intermediates that causes tissue damage. MDA is a low molecular weight end product formed via the decomposition of certain primary and secondary lipid peroxidation products.^[4] Numerous studies regarding the estimation of the lipid peroxidation by MDA levels are present and the role of the antioxidants in reducing the extent of peroxidation is being investigated. The present study is carried out to know the levels of lipid peroxidation in potentially malignant disorders by serum MDA analysis and whether administration of antioxidants will reduce the extent of lipid peroxidation.

Materials and Methods

The study was conducted in the Department of Oral Medicine and Radiology. The total number of patients included in the study were 30. Age groups of 30-60 years who had the habit of tobacco consumption who did not have any underlying systemic diseases or were not on any antioxidants were considered. Patients having any underlying systemic diseases or were on any antioxidants were not included in the study. After obtaining an informed consent, patients were examined thoroughly and a detailed case history of the individuals was recorded. Potentially malignant disorders were confirmed histopathologically. At the first appointment serum MDA was analyzed and these patients were strictly counseled to quit the habit and were prescribed with oral antioxidant tablets that consisted of beta-carotene - 10 mg, zinc sulfate monohydrate - 27.45 mg, monohydrated selenium dioxide

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- 70 mcg, manganese - 2 mg, copper - 1 mg, vitamin A, and vitamin C. One tablet three times a day was prescribed for a period of 2 months. The evaluation was done after 4 weeks of continuous treatment along with cessation of the tobacco chewing habit in the study group.

The patients were divided into the following groups:

First group (C1): 15 healthy individuals who are not having any habit of tobacco consumption.

Second group (S1): 15 patients with clinically and histopathologically diagnosed with potentially malignant disorders.

Third group (S2): 15 patients of the second group who were evaluated post-antioxidant treatment.

Methods

From the individual 2 mL of blood was collected. Serum was separated by centrifuging the blood sample at 3000 rpm for 5 min. Following which the serum MDA was measured using the method of Buege (1978). Serum-100 μ L serum is diluted to 500 μ L with distilled water. The samples are kept in boiling water bath for 15 min. To the diluted sample 1 mL of Trichloroacetic acid TCA-2-thiobarbituric acid (TBA)-HCl reagent is added. The reaction mixture is cooled and centrifuged. The supernatant is taken and the optical density of the pink color formed is read at 535 nm. The concentration of MDA in the sample is got by plotting the obtained absorbance against the standard graph. The optical density of the pink color formed is directly proportional to the concentration of serum MDA in the given sample.

Calculation

The optical density of the test samples is directly proportional to the concentration of MDA in the sample and calculated by the plotting against the standard graph and multiplied by the respective dilution factors the final concentration is expressed as μ M/L.

Results

The mean age of the group C1 was 47.46 ± 10.074 years and the mean age of group S1 was 39.50 ± 10.635 years. The gender distributions of the study groups were as follows group S1 comprised of 13 males and 2 females and group C1 comprised of 12 males and 3 females.

The mean serum MDA level in group C1 was 0.7900 ± 0.2336 μ M/L were as the mean serum MDA levels of group S1 was 2.478 ± 0.50756 μ M/L. The mean serum MDA of S2 was 2.160 ± 0.41252 μ M/L [Figure 1].

When the mean serum MDA levels of group C1 was compared to group S1 a highly significant difference was observed ($P < 0.0001$) using the unpaired *t*-test [Table 1]. When the mean serum MDA levels of C1 was compared to S2 using the same statistical test, a highly significant difference was observed. However, when

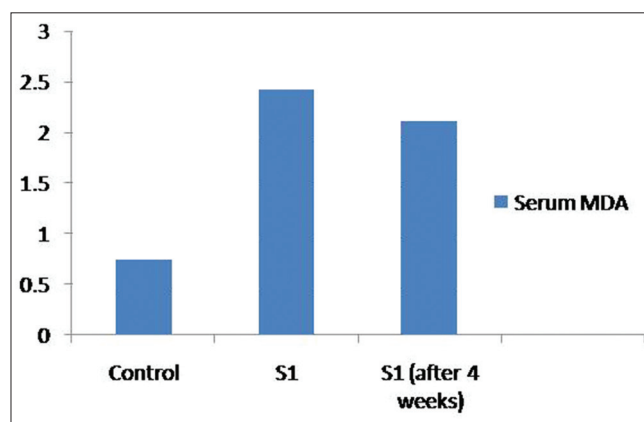


Figure 1: Mean serum MDA in control and study groups

an inter-comparison was done between group S1 and group S2 using paired *t*-test, a statistically significant difference was observed ($P < 0.05$) [Table 2].

When multiple comparisons were done among the groups using Honestly significant difference HSD Tukey's test, a statistically significant difference was observed among all three groups [Table 3].

Discussion

The habit of tobacco and betel nut chewing is a known etiological factor for the development of oral pre cancer and cancer in the head and neck.^[1] Tobacco cause the generation of free radicals and ROS which are responsible for the high rate of lipid peroxidation.^[2] Free radicals and ROS like hydrogen peroxide and hydroxyl radicals are highly reactive due to the presence of unpaired valence electrons.^[1] ROS leads to elevated lipid peroxidation, further damaging the cellular structural blocks like lipids, proteins, and DNA.^[3] Although lipid peroxidation of biological samples may be assessed by different chemical and physical methods, those based on the measurement of MDA formed from the breakdown of endoperoxides during the last stages of the oxidation of a Poly unsaturated fatty acids PUFA, appear as the most widely used.^[4] MDA is one of several low-molecular-weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products.^[5] Among the various methods to evaluate malondialdehyde, which include direct spectrophotometry or high pressure liquid chromatography, the reaction with thiobarbituric acid to form a colored adduct appears as a more rapid, inexpensive and sensitive technique.^[4] At low pH and elevated temperature, MDA readily participates in nucleophilic addition reaction with TBA, generating a red, fluorescent 1:2 MDA: TBA adduct. These facts, along with the availability of facile and sensitive methods to quantify MDA (as the free aldehyde or its TBA derivative), have led to the routine use of MDA determination in peroxidation.^[5] In our study of pre cancer groups, MDA level in potentially malignant disorders is at a higher level compared to normal individuals. This study found

Table 1: Inter-comparison of mean serum Malondialdehyde levels between controls and potentially malignant disorders

Groups (statistics)	Group	N	Mean	Standard deviation	Standard error mean	Significance
MDA ($\mu\text{M/l}$)	C1	15	0.7900	0.26336	0.06800	0.000
	S1	15	2.4787	0.50756	0.13105	

Unpaired *t*-test ($P < 0.001$); MDA: Malondialdehyde**Table 2: Inter-comparison of mean serum Malondialdehyde levels between group S1 and S2**

Groups	Mean	N	Standard deviation	Standard error mean	Significance
Pair 1					
MDA (S1)	2.4787	15	0.50756	0.13105	0.010
MDA (S2)	2.160	15	0.4782	0.1235	

Paired sample test ($P < 0.05$)**Table 3: Multiple comparison between the groups**

C1	0.7900
S1	2.4787
S2	2.1600

HSD Tukey's test

that the degree of extent of potentially malignant disorders is inversely proportional to the extent of lipid peroxidation.

According to Gupta *et al.*, MDA levels in oral submucous fibrosis were ($3.3 \pm 0.4 \text{ nmol/mL}$) were more compared to the healthy controls ($2.4 \pm 0.001 \text{ nmol/mL}$).^[6] According to Revant *et al.*, serum MDA was found to be $5.107 \pm 2.32 \text{ nmol/mL}$, whereas it was $9.33 \pm 4.89 \text{ nmol/mL}$ in oral precancer.^[1] Further an attempt was made to study the gender related changes in potentially related malignant disorders. The body contains a number of protective antioxidant mechanism, whose specific role is to remove harmful oxidants as they form, or to repair the damage caused by the reactive oxygen species.^[7] Antioxidant protection is never 100% efficient; thus, mechanisms of repair are of key importance for survival. When pro-oxidants increase or antioxidants fail, a situation of oxidative stress ensues that leads to excessive molecular damage and tissue injury.^[8] For example, antioxidant protection can operate at several different levels within cells by (a) preventing radical formation; (b) intercepting radicals when formed; (c) repairing oxidative damage caused by radicals; (d) increasing the elimination of damaged molecules; and (e) not repairing excessively damaged molecules to minimize the introduction of mutations.^[9] Dietary substitutes like beta carotene, provitamin A, vitamin A, vitamin C, vitamin E, lipoic acid, zinc, selenium, and spirulina can prevent oral cancer at a very early stage, i.e., in premalignant lesions, in compounds that can deactivate the free radicals and prevent their formation.^[10] Recent studies have suggested that these antioxidant nutrients act to inhibit the development of cancer cells and to destroy them through apoptosis (programmed cell death), by their stimulation of cytotoxic cytokines, by their action on gene expression, by preventing the development of

tumor's necessary blood supply or by cellular differentiation.^[11] According to Gitanjali *et al.*,^[12] and Gupta *et al.*,^[6] administration of antioxidant vitamin in cancer patients has shown that the administration of antioxidant in cancer patients, decreased the MDA level. Antioxidants may be able to cause the regression of premalignant lesions or inhibit their development into cancer. Preliminary studies have indicated that some antioxidants, particularly beta-carotene, may be of benefit in the treatment of precancerous conditions such as oral leukoplakia, which may be a precursor to oral cancer.^[13] Timely diagnosis of patients and administration of antioxidants has proven to be effective in declining the ROS and thus reducing the extent of damage on the cells. MDA may serve as a diagnostic tool in the estimation of oral pre-cancer and in evaluation of post-treated cases.

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